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Intracellular Physiology of the Rat Suprachiasmatic Nucleus: Electrical Properties, Neurotransmission, and Effects of Neuromodulators (Attachment 1-9)

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Our primary aim has been to study the electrophysiology of suprachiasmatic nucleus (SCN) neurons, with a focus on the interplay between intrinsic electrophysiological properties, amino-acid-mediated synaptic transmission, and neuromodulation. We have continued to study the role of excitatory and inhibitory amino acids (i.e., glutamate and GABA) in fast synaptic transmission in the SCN. Our work has provided strong evidence that these transmitters mediate all, or nearly all, of the fast synaptic potentials in virtually all SCN neurons. Preliminary experiments, however, suggest that a circadian rhythm of electrical activity persists after post-synaptic pharmacological blockade of these transmitter systems. Intracellular and whole-cell patch-clamp studies are being undertaken on intrinsic membrane properties, which we have found to be heterogeneous across the SCN. Particularly interesting is our recent observation that synchronous bursts of action potentials can occur in the SCN after chemical synapses have been blocked with low-calcium solutions and amino-acid-transmitter antagonists. Finally, we have continued several lines of experimentation partially supported by this grant on the supraoptic and paraventricular nuclei and the preoptic area of the hypothalamus, thus allowing a direct comparison between the SCN and other major regulatory areas of the hypothalamus. Our experiments continue to be aimed at providing a rigorous understanding of how transmitters and neuromodulators interact with intrinsic membrane properties to regulate the electrical activity of neurons in the SCN and other areas of the hypothalamus.

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13 JAN 1992

P.I.-F.E. Dudek
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1. RESEARCH OBJECTIVES

Our aim in this research project has been to test several important hypotheses about the electrophysiology of the suprachiasmatic nucleus (SCN). To accomplish this aim, we have used the hypothalamic slice preparation and several electrophysiological techniques (i.e., extracellular single- and multi-unit recording, intracellular recording, and whole-cell patch clamp). Our original project under support of this grant was to test the hypothesis that excitatory amino acids, in particular glutamate, are the transmitter system mediating the retinal input to the SCN. We have also tested the hypothesis that local GABAergic neurons mediate fast inhibitory input to SCN neurons by acting on $GABA_A$ receptors and by increasing a chloride conductance. More recently, we have focused on the intrinsic electrophysiological properties, and have tested the hypothesis that they are homogeneous across neurons in the SCN. We have attempted to identify distinct groups of cells with different electrophysiological properties, and in particular test for the presence of low-threshold calcium spikes and inward rectification. Another set of experiments has tested for a role for amino acid transmitters in generation of the circadian rhythm of electrical activity observed in the SCN; these experiments also provided interesting preliminary data concerning non-synaptic mechanisms of synchronization in the SCN. Thus, several interrelated projects, directly in line with our original proposal, are underway to study the electrophysiology of SCN neurons.

In addition to the work on the SCN, we have used this Air Force grant to partially support electrophysiological studies on other hypothalamic nuclei. Some of these experiments are continuing and relate directly to the experiments on the SCN, since our fundamental goal is to understand how the SCN compares electrophysiologically to other hypothalamic nuclei.

2. STATUS OF RESEARCH

A. Suprachiasmatic nucleus (SCN)

(i) Intracellular electrophysiology

(a) Excitatory amino acids

An extensive series of electrophysiological experiments on this topic with intracellular recordings were described in the previous progress report. Our studies provided direct evidence that both non-NMDA and NMDA receptors mediate synaptic transmission from both retinal inputs and other CNS sites. These experiments also showed that NMDA receptors are important in synaptic transmission when the SCN neurons are depolarized. A paper on this work was published in the *Journal of Physiology* (Kim and Dudek, 1991), and a copy is enclosed.

(b) Gamma-amino-butyric acid (GABA)

Another series of experiments have been aimed at studying inhibitory synaptic mechanisms

in the SCN. During our last progress report, we had obtained preliminary data and submitted an abstract (Kim and Dudek, 1990) reporting that spontaneous and evoked fast IPSPs were blocked by bicuculline, a $GABA_A$ receptor antagonist. During the last year these experiments were corroborated and extended, and a manuscript has been submitted for publication (copy enclosed). These data provide evidence that SCN neurons receive extensive GABAergic input and that $GABA_A$ receptors and an increase in chloride conductance mediate this synaptic mechanism.

(c) Membrane properties

More recent studies have been aimed at evaluating whether the electrophysiological properties of SCN neurons are homogeneous or heterogeneous, and if heterogeneous, whether distinct classes of neurons could be identified. We focused on the subpopulation of neurons that demonstrably received retinal input, as determined by recording short-latency EPSPs to optic nerve stimulation (Kim and Dudek, 1991). Considerable effort in the last year has been spent on a quantitative analysis of the electrophysiology of these SCN neurons with sharp and patch electrodes. A manuscript is in preparation on the experiments with sharp electrodes, and the work with patch electrodes is in various stages of completion. The experiments with sharp electrodes have indicated that individual action potentials are relatively short in duration, and are followed by a pronounced hyperpolarizing afterpotential. Spike inactivation, spike broadening and frequency accommodation occurred consistently during depolarizing current pulses, and an after-hyperpolarization routinely followed a burst of action potentials. The membrane time constant of these neurons ranged from 7 to 21 msec (mean 11.4 $\pm$0.7 msec). The input resistance of these neurons ranged from 105 to 626 megohms (mean 301 $\pm$23 megohms) with sharp electrodes. Although there was some variability in these properties, no distinct groups were found when analyses were made across the neuronal population. However, some neurons did show slight time- and voltage-dependent inward rectification, and these neurons had a higher spontaneous firing rate and were more excitable. In addition, some neurons had low-threshold calcium spikes (approx. 70% of the population), although other neurons clearly lacked them. These results suggest that: (1) SCN neurons receiving optic nerve input are not electrophysiologically homogeneous, and yet they do not form clear, distinct classes of electrophysiological cell types, (2) time-dependent inward rectification and the capacity to generate low-threshold calcium spikes are limited to only a sub-population of neurons, and (3) inward rectification is associated with an increased spontaneous firing rate. These electrophysiological properties have also been observed with whole-cell patch-clamp techniques, and voltage-clamp studies on these properties are being initiated.

(ii) Role of amino acid transmitters in the circadian rhythm of electrical activity in the SCN.

In our last progress report, we outlined preliminary experiments aimed at our long-term objective of understanding the possible role of glutamate and GABA in the expression of the circadian rhythm of electrical activity in the SCN. Yona Bouskila, a graduate student in our laboratory, had developed a procedure for using multi-unit extracellular recordings to study the circadian rhythm of electrical activity. This technique has worked well, and has the advantage that it is less labor-intensive because one does not have to continuously search for single-unit action potentials during an experiment to sample the population. Furthermore, it is more objective because

it removes any possibility of experimental bias in obtaining recordings from cells. We undertook a series of experiments examining the effects of excitatory and inhibitory amino acid receptor antagonists on the circadian rhythm of electrical activity, and our preliminary data suggested that these antagonists did alter the circadian rhythm of activity. However, we have undertaken numerous future experiments, and our data now appear to indicate that there is a circadian rhythm of multi-unit activity even when these transmitter receptors are blocked pharmacologically. More work is needed, however, because we have so far only recorded for slightly more than one circadian day. In additional experiments, we wish to extend the duration of our recordings so that we can evaluate two or three days of electrical activity. We also intend to work with animals whose circadian rhythm has been shifted by 12 hours, so that if we perform the experiment at the same time of the day, we can control for any other variables that could generate this apparent circadian rhythm of electrical activity in our hypothalamic slices. Finally, these studies included some preliminary experiments with low-calcium solutions, which led to an exciting series of experiments concerning non-chemical-synaptic mechanisms of synchronization (see below).

(iii) Non-chemical-synaptic mechanisms of synchronization in the SCN

Several independent observations in the literature concerning the SCN and circadian rhythms have suggested that the electrical activity of SCN neurons can be synchronized by mechanisms that do not involve chemical synaptic transmission. We found that when hypothalamic slices were bathed in a low-calcium solution for several hours, bursts of electrical activity occurred. It is particularly interesting that the bursts of activity are *synchronized* across the neuronal population. Multi-unit recordings showed that populations of SCN neurons have their bursts of activity roughly synchronized, and dual recordings from adjacent areas confirmed that in one SCN the bursts occurred synchronously across the population. However, the bursts in one SCN were not synchronized with bursts in the contralateral SCN. Furthermore, a mixture of NMDA, non-NMDA, and $GABA_A$ receptor antagonists had no effect upon the synchronicity of the bursts. Whole-cell patch-clamp recordings confirmed that the low-calcium solution blocked the evoked EPSPs and IPSPs, and the mixture of antagonists blocked the remaining spontaneous PSPs. These results indicate that synchronous neuronal activity can occur in the SCN without active chemical synapses, thus strongly suggesting that a different mechanism of communication exists in the SCN. The possible mechanisms include electrotonic coupling via gap junctions among SCN neurons, ephaptic interactions, and shifts in the concentration of extracellular ions such as potassium. Yona Bouskila and I are currently preparing a manuscript on these preliminary findings.

B. Other hypothalamic regions

(i) Paraventricular nucleus (PVN)

In our last progress report, we described several studies that had been undertaken on the electrophysiology and anatomy of PVN neurons. These studies have been published and are listed in the publication section, and reprints are enclosed. In essence, these studies showed that distinct electrophysiological properties are associated with anatomically specific cell types in the PVN (Tasker and Dudek, 1991; Hoffman, Tasker and Dudek, 1991). Selective non-NMDA receptor antagonists effectively blocked EPSPs in all of these different types of PVN cells and in neurons in the arcuate

nucleus. When combined with earlier work with Gribhoff in our laboratory and with van den Pol at Yale, the data provide strong evidence that glutamate is the dominant excitatory neurotransmitter in the hypothalamus (van den Pol, Wuarin and Dudek, 1991). More recent studies have shown that these different types of neurons have NMDA receptors that mediate synaptic transmission when the cells are depolarized (Wuarin and Dudek, 1991). Reprints of these papers are enclosed.

(ii) Supraoptic nucleus (SON)

Using whole-cell patch clamp techniques with the hypothalamic slice preparation, we have re-examined synaptic mechanisms in the SON. Our earlier work supported by this grant provided electrophysiological evidence that glutamate acting on non-NMDA receptors mediates fast synaptic transmission in this nucleus. We have confirmed with the more sensitive technique of whole-cell patch recording that all of the fast EPSPs and IPSPs appear to be mediated by glutamate and GABA. In the future, we hope to test this hypothesis more rigorously by evaluating whether nicotinic cholinergic receptors play a role in synaptic events in the SON.

(iii) Preoptic area

In our previous progress report, we outlined electrophysiological and anatomical studies in the preoptic area of the hypothalamus. We tested the hypothesis that this anatomically heterogeneous population of neurons was electrophysiologically heterogeneous. An extensive series of intracellular electrophysiological experiments have now demonstrated that virtually all the cells have low-threshold calcium spikes and linear current-voltage relations. Additional evidence has been accumulated that glutamate and GABA mediate synaptic events here, as in other hypothalamic regions. A manuscript on this work has been submitted, and whole-cell patch-clamp recordings are underway to evaluate these issues more rigorously.

3. PUBLICATIONS

Refereed Publications

Dudek, F.E., Obenaus, A. and Tasker, J.G. (1990) Osmolality-induced changes in extracellular volume alter epileptiform bursts independent of chemical synapses in the rat: importance of non-synaptic mechanisms in hippocampal epileptogenesis. Neurosci. Lett. 120:267-270.

van den Pol, A.N., Wuarin, J.P. and Dudek, F.E. (1990) Glutamate, the dominant excitatory transmitter in neuroendocrine regulation. Science 250:1276-1278.

Tasker, J.G. and Dudek, F.E. (1991) Electrophysiological properties of neurones in the region of the paraventricular nucleus in slices of rat hypothalamus. J. Physiol., London 434:271-293.

Wuarin, J.P. and Dudek, F.E. (1991) Excitatory amino acid antagonists inhibit synaptic responses in the guinea pig hypothalamic paraventricular nucleus. J. Neurophysiol. 65:946-951.

Hoffman, N.W., Tasker, J.G. and Dudek, F.E. (1991) Immunohistochemical differentiation of electrophysiologically defined neuronal populations in the region of the rat paraventricular nucleus. J. Comp. Neurol. 307:405-416.

Tasker, J.G., Hoffman, N.W. and Dudek, F.E. (1991) A comparison of three intracellular markers for combined electrophysiological, anatomical and immunohistochemical analyses. J. Neurosci. Meth. 38:129-143.

Kim, Y.I. and Dudek, F.E. (1991) Intracellular electrophysiological study of suprachiasmatic nucleus neurones in rodents: excitatory synaptic mechanisms. J. Physiol., London 444:269-287.

Manuscripts submitted

Kim, Y.I. and Dudek, F.E. Intracellular electrophysiological study of suprachiasmatic nucleus neurons in rodents: inhibitory synaptic mechanisms.

Hoffman, N.W., Kim, Y.I., Gorski, R.A. and Dudek, F.E. Intracellular membrane and synaptic properties in medial preoptic slices containing the sexually dimorphic nucleus of the rat.

Abstracts

Kim, Y.I. and Dudek, F.E. (1991) Electrical heterogeneity of suprachiasmatic nucleus (SCN) neurons that receive optic nerve input. Soc. Neurosci. Abstr. 17:669, #263.10.

4. PROFESSIONAL PERSONNEL

Mr. Yona Bouskila
Dr. Victor Corvalan
Dr. Neil H. Hoffman
Dr. Yang I. Kim
Dr. Jean-Pierre Wuarin

5. INTERACTIONS

At the 1990 Winter Conference on Brain Research, Drs. E. Dudek, M. Gillette, M. Rea and A. van den Pol participated in a workshop on the SCN. We all then attended the 1991 meeting and have several informal discussions on our research. I have proposed, and Dr. Haddad has supported, that we hold a one-day workshop to discuss our latest work on this topic, possibly at the USAF School of Aerospace Medicine in San Antonio. I have spoken with all of the participants, and will arrange a proposed date within the next few weeks.

6. NEW DISCOVERIES, INVENTIONS OR PATENT DISCLOSURES--none, other than the research findings described below

7. OTHER STATEMENTS

During the next year or two, we will focus on local synaptic interactions, using glutamate microstimulation and whole-cell patch-clamp electrophysiology to test the hypothesis that *local* GABAergic neurons generate the fast IPSPs to SCN neurons. In addition, we will modify our slice preparation (possibly use sterile techniques, etc.) to obtain recordings that last for 2-3 days so that we can test more rigorously the hypothesis that the circadian rhythm of electrical activity in the SCN does not depend on amino-acid-mediated neurotransmission. Finally, several possible experiments are being considered to study non-chemical-synaptic mechanisms of synchronization.